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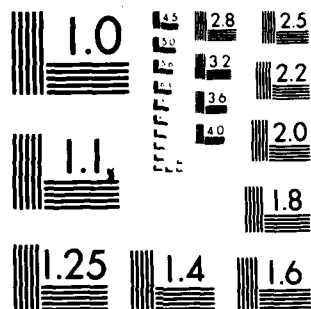
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BRAIN MICROCIRCULATION  
OBSERVED IN VIVO AFTER TRAUMA

ANNUAL REPORT

William I. Rosenblum, M.D.

October 31, 1978

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Brain Microcirculation Observed in Vivo After Trauma

Annual Report DAMD 17-76-C-6020

This years work was simply an extension "without cost". Therefore we had available only a limited number of dollars, not expended in the prior contract period. The major thrust of the years work was devoted to in vitro analysis of platelet aggregation, using mouse platelets. With these studies, we hoped to gain insight into the mechanisms underlying the complex phenomena which we observed during the first two contract years. The latter resulted in three major publications (1,2,3).

In addition to our in vitro work, additional in vivo studies were performed, all of which dealt with the response of cerebral microvessels to damage of adjacent brain. None of the data upon which the following summary is based, have yet been submitted for publication.

In Vitro Responses of Mouse Platelets:

There are almost no reports concerning the behavior of mouse platelets in vitro. We thought it important to examine their behavior since our in vivo model was a mouse model. In vitro and in vivo comparisons in the same specie would obviate the disadvantage seen in some published studies of others, wherein explanations for in vivo platelet behavior were drawn from in vitro studies of platelets from a different specie, with the attendant risk of erroneous conclusions.

We found that in platelet rich plasma (PRP) mouse platelets were aggregated by ADP, epinephrine, collagen, thrombin and arachidonic acid. ADP produced only one wave of aggregation, which was followed by deaggregation. The response to collagen, thrombin, or arachidonate was irreversible, and when conventional citrate was used as an anticoagulant, the response was a single wave of aggregation. When heparin was the anticoagulant used, collagen induced two waves of aggregation in PRP, and a similar response was sometimes seen with arachidonate. When EDTA was used as the aggregating stimulus, a most surprising result occurred. Thrombin was still capable of producing aggregation. Moreover, rather than producing a single wave of aggregation, as seen with heparin, two waves of aggregation were now observed.

This result was surprising, because EDTA, by removing calcium, obliterates the response of platelets from other species. Indeed the response of mouse PRP to the other aggregants was abolished in EDTA. In fact, since the response of mouse platelets seemed more readily produced in heparinized rather than citrated PRP, and since two waves of aggregation could be produced by collagen in the former and not in the latter, it appeared that calcium removal hindered rather than helped the response of mouse platelets. Hence the continued response to

thrombin and the appearance of two waves in EDTA, seemed inconsistent with our other data and data from other species. However results with aggregate inhibitors described below, suggested not only that synthesis of cyclic endoperoxides, prostaglandins and/or their derivatives was related to the aggregation of mouse platelets, as has been suggested for other species, but also that such synthesis was occurring when thrombin was added, even in the presence of EDTA.

#### Inhibition of Aggregation in Vitro

Aspirin and indomethacin are each inhibitors of cyclo oxygenase and hence prevent conversion of arachidonate to prostaglandins and/or cyclic endoperoxides and their derivatives. Both aspirin and indomethacin prevented arachidonate from aggregating heparinized PRP. This was so when aspirin or indomethacin were added to the cuvette and also when aspirin or indomethacin were given intraperitoneally one hour before harvesting the platelets. Appropriate mixing experiments showed that the effect of intraperitoneal aspirin was not due to residual aspirin in the plasma of the PRP. Unlike its effect on the response to arachidonate, aspirin, when added to the cuvette, failed to inhibit the response of heparinized PRP to collagen, unless large (100-500  $\mu\text{g/ml}$ ) concentrations of aspirin were present, and heparinized platelets harvested from aspirin treated mice had normal responses to collagen. When collagen produced two waves of aggregation in heparinized PRP, the addition of large doses of aspirin abolished the second, but not the first wave. Similarly, large amounts of aspirin in the cuvette, abolished the second, but not the first wave of aggregation produced by thrombin in EDTA.

Aggregation produced by arachidonate was also inhibited by addition of prostacyclin ( $\text{PGI}_2$ ) to the cuvette. Moreover, while addition of up to about 10mM arachidonate produces increasingly large, and finally maximal, irreversible aggregation in heparinized PRP, progressively higher concentrations of arachidonate produce increasingly reversible aggregation and then smaller overall aggregation as well as reversibility. This suggests that arachidonate is converted to both an aggregation producing and an aggregation inhibiting agent by the mouse platelet. The latter could be  $\text{PGI}_2$ , whose production in effective amounts might depend upon the addition of very large amounts of arachidonate substrate, and whose presence might be unmasked by the passage of time in the cuvette which would permit shorter lived aggregating agents to disappear and the aggregation to then reverse in the remaining  $\text{PGI}_2$ .

#### Relation of In Vitro Events to In Vivo Events

We previously reported that induction of aggregates in cerebral microvessels was inhibited by aspirin and indomethacin given intraperitoneally. The in vitro results presented above show that similar phenomena are seen in vitro, but do not permit one to suggest the in vivo sequence of chemical events, or whether the mechanism by which aspirin and indomethacin worked was identical in the in vitro and in vivo situation. A unifying hypothesis would suggest that our in vivo

methods of inducing aggregation somehow activate transformation of arachidonate to other substances and that blockade of this transformation, through inhibition of cyclooxygenase, blocks the aggregation. If this is so, our in vitro data indicates that exposure of collagen was not the means by which the arachidonate transformation was initiated in our model employing vascular injury, since aspirin was not a very effective inhibitor of collagen's effect, in vitro. The in vitro data also suggest that PGI<sub>2</sub> could be an effective in vivo inhibitor of aggregation as has been suggested for the platelets of other species.

#### Additional In Vivo Studies

As noted in the first paragraph of this report, the major thrust of this "No-cost" year has been in vitro studies. Nevertheless one in vivo study was also carried out. This study employed a model in which we stabbed the brain with a needle and examined adjacent, untouched vessels with vital microscopy. As published last year, this technique often produces platelet aggregates in venules. However, the incidence with which such aggregates is produced has proved highly variable, and so often falls to less than 20% of a given number of stabbed animals. As reported in last years progress reports, we performed large numbers of studies varying depth and number of punctures in an attempt to produce a more uniform model. The variability greatly reduces the value of this model because the influence of drugs, etc., cannot be readily evaluated if the aggregates are infrequently produced. In an effort to increase the reproducibility we performed the following study.

1 mM ADP was applied to the cerebral surface prior to stabbing the brain. No aggregates were produced. The surface was washed, artificial CSF replaced the ADP and the brain was stabbed. In this particular series of 10 mice, none displayed aggregates following stabbing. ADP was reapplied. Now aggregates appeared within three minutes, in two of the ten where no aggregates had previously been seen.

This study was stimulated by the knowledge that ADP alone can induce aggregates in damaged vessels when the damage itself was insufficient to produce visible aggregates. We thought that if brain puncture in some way "injured" vessels - as it must when aggregates are induced - then lesser degrees of the same injury might be revealed through the synergistic or additive effect of ADP. This indeed seemed to be the case. We must stress that the observed vessels were not touched by the needle and hence were not directly injured by it (see our previous publications). A main thrust of the renewal proposal which follows is to continue our attempts to increase the yield of aggregates in untouched vessels following brain injury, and to develop testable hypotheses concerning the nature of the "injuring" substances released from damaged brain by analysis of the nature of those materials potentiating the injury. In addition we will study the effects of tissue injury on adjacent untouched vessels in a peripheral (i.e. extracerebral) vascular bed.

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